SUN YAT-SEN UNIVERSITY,

ZHONGSHAN SCHOOL OF MEDICINES

APPLICATION FORM FOR ETHICAL REVIEW OF WELFARE AND ETHICS OF EXPERIMENTAL ANIMALS

<table>
<tr><td colspan="4">Protocol name: Tracing and modifying the homing capability of bone marrow derived mesenchymal stem cells</td></tr>
<tr><td colspan="4">Protocol source: General Program, The National Natural Science Foundation of China</td></tr>
<tr><td colspan="2">Principle investigator:</td><td colspan="2">Name of unit: Key Laboratory for Stem Cells and Tissue Engineering</td></tr>
<tr><td colspan="2">Title: Professor</td><td>Tel: +86 13543445898</td><td>E-mail: xiangp@mail.sysu.edu.cn</td></tr>
<tr><td colspan="4">Experimental animal using permission No.: SYXK (粤) 2015-0107</td></tr>
<tr><td colspan="4">Experiment schedule: July 1st, 2016 to October 1st, 2016</td></tr>
<tr><td colspan="4">Please provide the name and the permission number of previous censored projects and elaborate which are similar or identical to this project.
None</td></tr>
<tr><td colspan="4">Is the project correlate to previous censored projects? If so, write down the project name and the approval number.
No</td></tr>
<tr><td rowspan="2">Experimental animal</td><td>Source:
Sun Yat-sen experimental animal center</td><td colspan="2">Breed strain: BALB/c mice</td></tr>
<tr><td>Level: SPF (√)
Normal ( )</td><td colspan="2">Number of BALB/c mice: 42
(♂ 42)</td></tr>
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The purpose, method, significance and feasibility of the experiments

Purpose:

Separate, identify and engineer bone marrow derived mesenchymal stem cell (MSC) in order to empower the homing ability of MSC to local inflammatory sites of inflammatory bowel disease (IBD) and mark MSC with fluorescent protein for tracing.

Method:

1. EF-1α-CXCR4-T2A-luciferase-IRES-eGFP plasmid and EF-1α- luciferase-IRES-eGFP plasmid will be constructed and transfected respectively by lentivirus to human MSCs.

2. $MSC^{EF\text{-}1\alpha\text{-}luciferase\text{-}IRES\text{-}eGFP}$s that simultaneously express eGFP and MSC $^{EF\text{-}1\alpha\text{-}CXCR4\text{-}T2A\text{-}luciferase\text{-}IRES\text{-}eGFP}$ expressing CXCR4 and eGFP will be screened out by flow cytometry

3. We select the most suitable temperature (24℃) for the mice. The light is operated as the circadian rhythms and the noise and vibration will be well controlled. Day 1, carefully shave a 1cm×1cm filed on the back between the shoulders of the mouse using an electric razor. While holding the mouse with one hand, smear the shaved abdominal skin with presensitization solution (150ul/20g), which is the mixture of acetone, olive oil and TNBS in 16:4:5 scale. Control mice are treated with presensitization solution without TNBS. Leave the mice until day 7. On day 7, absolute diet is applied to the mice apart from the water. One day 8, anesthetize the mouse by intraperitoneal injection of 4% chloral hydrate (150ul/20g). Insert the catheter into the colon 4cm proximal to the anus and slowly administer 150ul/20g TNBS/alcohol solution into the colon. Keep the mice with hand down for 60s before returning to the cage. Concerning the damaged colon, we take the jelly for the mice to lessen the pain after modelling. The number of the mice per cage decrease from 5 to 2 after modelling and the packing will be altered every 3 days.

4. Weight the mouse after day 7 every morning and assess the mice as the standard below:

Score	Weight lost (%)	Stool form	Hematochezia
0	0	Normal	Normal
1	1-	Loose(+)	Fecal occult blood(+)
2	5-	Loose(+ +)	Fecal occult blood(+ +)
3	10-	Liquidness(+)	Hematochezia (+)
4	>15	Liquidness(+ +)	Hematochezia(+ +)

. 5. On day 9, animals with established colitis will receive $MSC^{EF\text{-}1\alpha\text{-}luciferase\text{-}IRES\text{-}eGFP}$, MSC $^{EF\text{-}1\alpha\text{-}CXCR4\text{-}T2A\text{-}luciferase\text{-}IRES\text{-}eGFP}$ and saline. On day 11, mice will be respectively euthanized by cervical dislocation of spine. The colon length, DAI score and the concentration of the cytokines will be performed using SPSS.

All the animals will be sacrificed by cervical dislocation. Once the mice are in great pain, they will be sacrificed to reduce the suffering. The process of feeding, operation and management are in conformity with the detail rules for the implementation of laboratory biological safety management and experimental animal welfare ethics principle.

Significance:

Without involving ethic problems in clinical application, with a handful of side effect or concern of safety, MSC is playing an important role in immuno-regulation and immune-reaction such as GVHD, autoimmune disease and so on. However, due to lack of chemokine receptors, MSC is blamed for its low homing efficiency to inflammatory sites that in several cases MSC failed to live up to its expected effect. Meanwhile, limited facts about the in vivo distribution of intravenous injected MSC have been testified because of the difficulty to localize in living body. Thus, empowering MSC with chemokine receptors and maker molecules to enhance their ability of homing and immune-regulation not only contributes to improve the clinical efficacy as well as the prognosis of patients, but also sharpens the understanding of the distribution of intravenous injected MSC in vivo.

Feasibility:

1. Full foundation.

Our project is based on sufficient article reading and clinical information which prop up the evidence of chemokines of specific organs, the immuno-regulation function of MSC, the positive outcomes of both implement in patients and disease models of mouse and the in body marking function of Luciferase.

2. Lab.

The lab condition and staff guarantee the actualization of the project. Our research is supported by Key Laboratory for Stem Cells and Tissue Engineering which is a center for Stem Cell Biology and Tissue Engineering, supported by Zhongshan School of Medicine, Sun Yat-sen University, Ministry of Education. With a Safety Level 2 laboratory of approximate 3500m^2 and equipment worth over 15 million, we are equipped with important and supportive platforms, such as stem cells bank and stem cell preclinical primate evaluation platform, as well as necessary equipment for the project such as BD-Influx flow cytometry、 Illumina Solexa GAIIX gene sequencing instrument and laser scanning confocal microscope.

Feeding situation of experimental animals	Fed and managed by Sun Yat-sen experimental animal center
The design of the animal experiment	Experimental grouping: IBD MODEL（♂ 30）: IBD model with 30 BALB/c mice will be established and divided into 3 experiments: (1) 10 mice will be included in this experiment. After sensitization, 10 mice will be injected with MSC$^{EF\text{-}1\alpha\text{-}CXCR4\text{-}T2A\text{-}luciferase\text{-}IRES\text{-}eGFP}$ (2) 10 mice will be included in this experiment. After sensitization, 10 mice will be injected with MSC$^{EF\text{-}1\alpha\text{-}luciferase\text{-}IRES\text{-}eGFP}$. (3) 10 mice will be included in this experiment. After sensitization, 6 mice will be injected with saline. Control group（♂ 12）: (5) 6 mice will be treated with alcohol for the enema as the alcohol control. (6) 6 mice without any further treatment will be as the normal control group.
	Administration mode: intragastric administration ( ) subcutaneous injection ( ) intraperitoneal injection ( ) intravenous injection (√) others ( ) Note if 'other' is chosen: Enema
	Executing manner: cervical dislocation (√) overdose of anesthetic ( ) others ( ) Note if 'other' is chosen:
	The management of dead bodies: sanity treatment (√) others( ) Note if 'other' is chosen:

The embodiment of "3R" principle in designing the experiments	Replacement: In our design, we choose to culture bone marrow derived mesenchymal stem cell in vitro to replace living animals.
	Reduction: In our design, we have reduced the number of animals used. We will establish IBD model of BALB/c mice which will be divided into 5 experiments. In the first three experiments will injected $MSC^{EF\text{-}1\alpha\text{-}luciferase\text{-}IRES\text{-}eGFP}$, $MSC^{EF\text{-}1\alpha\text{-}CXCR4\text{-}T2A\text{-}luciferase\text{-}IRES\text{-}eGFP}$ and saline to mice after modeling. The 4^{th} group will be treated with alcohol for the enema and the normal control group will not receive any further treatment. In the following experiment, The colon length, DAI score and the concentration of the cytokines will be performed using SPSS.
	Refinement: All the experiment technique required in our project, such as anesthesia, surgical operations and function test has been fully mastered. At the same time, the experimental technical route has been delicately designed and selected in order to reduce discomfort and suffer of experimental animals which will promise the repeatability of animal experiment.

Announcement: I will consciously abide by the experimental animal welfare ethics principle and agree to accept the supervision as well as inspection of commission and inspector of laboratory manager. If I violate the commitment or stipulation, I will voluntarily accept punishment.

Signed (or stamped) by the announcer: Zhou Longyuan Date: 1st, August, 2016

Review opinion of the declaring unit:

Agree.

Signed (or stamped) by the head executive investigator:

Date 10th, August, 2016

Instruction:

1. The principle and the head executive investigators of the project and the leader of cooperative departments are required to sign in the announcer's signature column.
2. materials for review shall be submitted with the application (a copy of electronic as well as written document). The issues involving animal welfare and ethics, such as significance, necessities, purpose and feeding management of experimental animal, the experiment operation, observation steps and methods need expatiation. Additional pages are acceptable.
3. Please print double-sided.